SOME FACTORS INFLUENCING THE FINAL HYDROGEN-ION CONCENTRATION IN BACTERIAL CULTURES WITH SPECIAL REFERENCE TO STREPTOCOCCI

Horry Jones

From the Department of Pathology and Bacteriology, University of Illinois, College of Medicine, Chicago

It is a well known fact that a given organism in fermenting a utilizable carbohydrate, produces a gradual alteration in the reaction of the medium, and that this change in reaction eventually becomes so marked as to inhibit all further growth of the organism. The degree to which this alteration in reaction proceeds in a given culture is designated as the limiting or final hydrogen-ion concentration of that organism.

In some instances, accurate measurements of this final hydrogen-ion concentration have shown it to be one of the most remarkably constant characteristics, not only of a given organism, but of various strains of this organism. In fact, following the researches of Sorensen,[1] of Michaelis,[2] and later of Clark and Lubs,[3] the application of hydrogen-ion concentration determinations has come into quite prominent use in the differentiation of certain strains of bacteria from other strains more or less closely allied. For example, Clark and Lubs have shown that in the presumptive test for B. typhosus, an organism which, by the usual tests, is indistinguishable from B. coli of human fecal origin, is often encountered. They have shown, also, that this organism is not of human fecal origin, and that it may be differentiated from B. coli on the basis of differences in their final hydrogen-ion concentrations, when grown for five days in glucose broth.

In a similar way, Ayers[4] and others have shown that virulent hemolytic streptococci found in milk and other sources may be differentiated from nonvirulent hemolytic strains by differences in their final hydrogen-ion concentrations. Avery and Cullen[5] repeated this work with similar results.

Received for publication Oct. 13, 1919.

[1] Ergebn. Phisiol., 1912, 12, p. 303.
[2] Wasserstoffionenkonzentration, 1914.
[3] Jour. Infect. Dis., 1915, 17, p. 160; Jour. Bacteriol., 1917, 2, p. 1.
[4] Jour. Infect. Dis., 1918, 23, p. 290.
[5] Jour. Exper. Med., 1919, 29, p. 215.

The present work was undertaken for the purpose of determining if successive transfers of a given organism in the same medium, incubated under the same conditions, would develop identical concentrations of hydrogen-ion, and if so, to study the effect of some of the commoner factors, such as temperature, initial reaction, composition of medium, etc., in causing deviations from that final hydrogen-ion concentration.

METHODS

The hydrogen electrode described elsewhere[6] was used in making these determinations. The readings are accurate to half a millivolt, or about 0.01 of a P_H. Four day periods of incubation were used unless otherwise stated.

EXPERIMENTAL

To make valid any interpretations as to the effect of varying conditions on the final hydrogen-ion concentration, it was of course necessary, first of all, to determine whether a given organism could, under identical environment, reproduce identical concentrations of hydrogen-ion. The results of this experiment are recorded in table 1.

TABLE 1

HYDROGEN-ION CONCENTRATION PRODUCED BY SUCCESSIVE TRANSFER OF A HEMOLYTIC STREPTOCOCCUS OF VIRULENT TYPE

No. of Transfer	Reading in Millivolts	P_H	Variations
1	576.5	5.11	0
2	576.5	5.11	0
3	576.5	5.11	0
4	576.0	5.10	.01
5	576.5	5.11	0

Incubated at 37 C. for 4 days, in broth containing 1% peptone, 0.3% beef extract, 0.7% NaCl, and 1% glucose.

According to these results, a most surprising consistency is shown by Str. hemolyticus in its limit of hydrogen-ion tolerance, from one transfer to the next. Other strains of Str. hemolyticus and cultures of B. typhosus, B. paratyphosus, and B. proteus showed practically the same regularity through five successive transfers.

EFFECT OF VARYING THE PERCENTAGE OF GLUCOSE IN A GIVEN MEDIUM

Varying percentages of sugar were added to ordinary broth, beginning with 0.1% and increasing up to 1%. Some variation in ability to

[6] Jones, H. M., Jour. Infect. Dis., 1919, 25, p. 262.

completely remove glucose from the medium was observed in the various organisms studied. B. paratyphosus alpha and B. proteus removed all of the 0.2% of glucose and the reaction of the medium then turned back to alkalinity. With percentages above 0.2%, the final hydrogen-ion concentrations for a given organism were identical in the various sugar concentrations. The amount of glucose which a given organism can consume is influenced by the buffer content of the medium, i. e., by such constituents as phosphates, protein, etc., which aid in holding the concentration of hydrogen-ion from the toxic limit, thus permitting a larger amount of the sugar to be decomposed. An initial reaction with a P_H well over on the alkaline side has a similar effect. Streptococcus hemolyticus of virulent type did not remove all of the 0.2% glucose, while a Str. hemolyticus obtained from milk removed 0.2% and subsequently developed an alkalinity.

These two factors of buffer content of the medium and percentage of glucose deserve consideration. Berman and Rettger,[7] in studying the proteolytic activity and nitrogen metabolism of various organisms, erred in the interpretations of their results through lack of consideration of both of these factors.

EFFECT OF VARYING THE KIND OF UTILIZABLE CARBOHYDRATE

Str. hemolyticus, pneumococcus, and B. coli were inoculated into 1% glucose and 1% lactose broths, and incubated for 4 days at 37 C.

TABLE 2
RESULTS OF EXPERIMENT

Organism	P_H in Glucose	P_H in Lactose
Str. hemolyticus	5.11	6.0
Pneumococcus, type 1	5.43	6.2
Pneumococcus, type 3	5.6	6.02
B. coli	4.31	4.33

To extend the experiment to include other organisms and other sugars would doubtless show that the more vigorous organisms are affected more by the toxicity of the hydrogen-ion than by the nature of the sugar. These results, however, are sufficient to show that it is necessary to specify the kind of sugar used in the medium when referring to the limiting hydrogen-ion concentration of some organ-

[7] Jour. Bacteriol., 1918, 3, p. 389.

isms; they also show that a carbohydrate which is utilizable under certain conditions may not be utilizable under other slightly more adverse conditions.

EFFECT OF ADDITION OF HUMAN BODY TO GLUCOSE BROTH

Certain organisms are grown with difficulty in ordinary glucose broth, and for this reason blood, blood serum, or ascitic fluid are frequently added to the medium to favor growth. How ascitic fluid in varying amounts affects the final hydrogen-ion concentration is shown in the following table.

TABLE 3

FINAL HYDROGEN-ION CONCENTRATION OF STR. HEMOLYTICUS OF VIRULENT TYPE IN GLUCOSE BROTH CONTAINING VARYING AMOUNTS OF ASCITIC FLUID

Composition of Medium	Millivolts	P_H
10 c c glucose broth = 3 c c ascitic..............	548.5	4.63
10 c c glucose broth = 2 c c ascitic..............	548.5	4.63
10 c c glucose broth = 1 c c ascitic..............	548.5	4.63
10 c c glucose broth = ½ c c ascitic.............	548.5	4.63
10 c c glucose broth = 3 drops ascitic............	548.5	4.63
10 c c glucose broth = no ascitic..................	576.5	5.11

From these results it is seen that, according to the work of Ayers and others referred to above, this organism when grown in 1% glucose broth would be classed as a streptococcus of virulent type because of its final hydrogen-ion concentration, but when grown in ascitic broth it would be classed as one of nonvirulent type, i. e., in ascitic broth its P_H is similar to that of a nonvirulent strain, while in plain broth its P_H is that of the virulent types. The final hydrogen-ion concentrations of different pneumococcus strains also show this same alteration when grown in ascitic broth. How the ascitic fluid brings about such a marked alteration in the tolerance of these organisms to the toxic hydrogen-ion is not clear. The phenomenon appears all the more puzzling when it is seen that the streptococci of nonvirulent types are not similarly affected by the presence of ascitic fluid in the medium. Furthermore, autoclaving the ascites broth reduces it to the condition of ordinary broth in this respect.

Delicately growing organisms, as Str. hemolyticus of virulent type and pneumococci of various type, showed wide variation even from one transfer to the next when subjected to conditions which tended to delay growth. For example, one strain of pneumococcus when inoculated into a medium whose initial reaction was P_H 7 grew poorly and

developed a final concentration of only P_H 6.2, but in a medium with an initial reaction of P_H 7.6, the growth was abundant, and a final concentration of P_H 5.4 was reached.

Another strain required a heavy inoculum before abundant growth could be obtained, and its final concentration of hydrogen-ion varied from one transfer to another depending on the abundance of growth secured. Incubating at room temperature and also the age of the culture from which the transfer was made have a similar effect on growth, and, likewise, on the final hydrogen-ion concentration. The statement often made that the final hydrogen-ion concentration of a given organism is always the same regardless of the initial concentration, provided growth occurs, is therefore not true, at least, with certain delicately growing strains of bacteria.

CONCLUSION

From these results it is seen that, to obtain any accurate information regarding the final hydrogen-ion concentration of an organism, a number of factors should be taken into consideration. This characteristic, the limiting hydrogen-ion concentration of a given organism, to have any significance or subsequent application should be defined in terms of the composition of the medium, the initial reaction and any other conditions which favor or hinder abundant growth of that organism.